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Thomas J. Reid III, Mark Mullaney, Linda M. Burrell, John Redmond III, Kenneth F. Mangan

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Acquired pure red cell aplasia (PRCA) has been associated with various lymphoproliferative conditions but its occurrence with Hodgkin's disease is rare. We report a case of PRCA occurring immediately following the completion of induction chemotherapy in a patient with Stage IIIB nodular sclerosing Hodgkin's disease. In vitro erythroid colony studies documented evidence for T cell mediated suppression of erythropoiesis and lack of a serum inhibitor. Addition of cyclosporin to the in vitro cultures stimulated erythroid colony growth. Following in vivo treatment with cyclosporin peripheral blood CD4/CD8 ratios returned to normal. However, serum erythropoietin levels were inappropriately low. Subsequent treatment with erythropoietin induced a reticulocytosis and transfusion independence. Since discontinuing the erythropoietin, the patient has been able to maintain a hemoglobin of 100 g/L. This case illustrates that red cell aplasia occurring in the setting of Hodgkin's disease may be due to T cell mediated suppression of erythropoiesis. A response to cyclosporin may be masked by inappropriately low erythropoietin levels.

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# Pure Red Cell Aplasia After Chemotherapy for Hodgkin's Lymphoma: In Vitro Evidence for T Cell Mediated Suppression of Erythropoiesis and Response to Sequential Cyclosporin and Erythropoietin

Thomas J. Reid III, Mark Mullaney, Linda M. Burrell, John Redmond III, and  
Kenneth F. Mangan

Department of Hematology, Walter Reed Army Medical Institute of Research, Washington, D.C. (T.J.R.); Department of Medicine, Temple University, Philadelphia, PA (M.M., K.F.M.); and Department of Medicine, Hematology-Oncology Service, Walter Reed Army Medical Center, Washington D.C. (L.M.B., J.R.)

Acquired pure red cell aplasia (PRCA) has been associated with various lymphoproliferative conditions but its occurrence with Hodgkin's disease is rare. We report a case of PRCA occurring immediately following the completion of induction chemotherapy in a patient with Stage IIIB nodular sclerosing Hodgkin's disease. In vitro erythroid colony studies documented evidence for T cell mediated suppression of erythropoiesis and lack of a serum inhibitor. Addition of cyclosporin to the in vitro cultures stimulated erythroid colony growth. Following in vivo treatment with cyclosporin peripheral blood CD4/CD8 ratios returned to normal. However, serum erythropoietin levels were inappropriately low. Subsequent treatment with erythropoietin induced a reticulocytosis and transfusion independence. Since discontinuing the erythropoietin, the patient has been able to maintain a hemoglobin of 100 g/L. This case illustrates that red cell aplasia occurring in the setting of Hodgkin's disease may be due to T cell mediated suppression of erythropoiesis. A response to cyclosporin may be masked by inappropriately low erythropoietin levels.

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**Key words:** Hodgkin's disease; red-cell aplasia, pure; cyclosporin A; erythropoietin; chemotherapy; immunoglobulins, intravenous

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## INTRODUCTION

Pure red cell aplasia is a syndrome characterized by hypoproliferative normochromic, normocytic anemia, reticulocytopenia, and selective erythroid hypoplasia with preservation of normal myelopoiesis and megakaryopoiesis [1]. A variety of diseases and clinical conditions have been associated with chronic acquired PRCA. The lymphoproliferative disorders are the neoplasms most commonly associated with pure red cell aplasia [2], especially chronic lymphocytic leukemia and the non-Hodgkin's lymphomas [3]. The occurrence of PRCA with Hodgkin's disease, however, is rare with only five previously reported cases [4-8]; in all cases, PRCA either preceded or was concomitant with the diagnosis of Hodgkin's disease. Antibody or T cell mediated suppression of erythropoiesis is responsible for PRCA occurring in lymphoproliferative disorders [9,10]. However, the mechanism for the development of PRCA in Hodgkin's disease is

obscure. We report a case of PRCA occurring after completion of chemotherapy in a patient with stage IIIB nodular sclerosing Hodgkin's disease. In vitro erythroid colony studies and in vivo response to sequential cyclosporin and erythropoietin was consistent with T cell mediated suppression of erythropoiesis. An initial response to cyclosporin was masked by suboptimal erythropoietin production.

## CASE REPORT

A 25-year-old female presented in January 1989 with a 6 month history of diffuse adenopathy, fevers, and night

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Address reprint requests to Dr. T.J. Reid III, Department of Hematology, Walter Reed Army Medical Institute of Research, Washington, D.C. 20307.

sweats. Physical examination revealed bilateral cervical, supraclavicular, and axillary adenopathy but no hepatosplenomegaly. A biopsy of a left cervical lymph node showed nodular sclerosing Hodgkin's lymphoma. Staging evaluations including CT of the chest, abdomen, and pelvis revealed bilateral hilar and massive retroperitoneal and pelvic adenopathy. An initial bone marrow aspirate and biopsy showed complete trilineage maturation in erythroid, myeloid, and megakaryocyte lines with no evidence of Hodgkin's disease. Initial blood counts revealed a hemoglobin 97 gm/L, reticulocyte count 0.7%, white blood cell count  $14.9 \times 10^9/\text{L}$  with a normal differential, and platelets of  $535,000/\text{mm}^3$ . A diagnosis of clinical stage IIIB Hodgkin's disease was made. No staging laparotomy or splenectomy was performed. The patient was treated with MOPP/ABV chemotherapy for six cycles (CALGB 8695) and a complete response was achieved after three cycles as judged by follow-up physical examination and CT scans. Three additional cycles of MOPP/ABV were administered and the patient completed her sixth course by July 1989. At that time, a complete blood count revealed hemoglobin 119 gm/L, white cell count  $9.5 \times 10^9/\text{L}$ , platelets of  $205,000/\text{mm}^3$ , and reticulocytes 0.8%. One month later, the patient became acutely ill with fever, acute sinusitis, *Pseudomonas aeruginosa* bacteremia, and *Pneumocystis carinii* pneumonia. She was treated with various antibiotics including erythromycin, vancomycin, piperacillin, mezlocillin, gentamicin, ampicillin, bactrim, pentamidine, cefuroxime, and ceftazidime. Following a prolonged hospitalization, the patient eventually recovered. At discharge, the white cell count was  $3.3 \times 10^9/\text{L}$  (normal differential counts) and the platelets were  $332,000/\text{mm}^3$ . However, the patient was profoundly anemic with a hemoglobin of 53 gm/L and a reticulocyte count of 0.1%. There was no evidence of gastrointestinal bleeding. The anemia persisted and she became transfusion dependent; postransfusion hemoglobins were  $\leq 10.8 \text{ g/dL}$ . All medications were discontinued. A bone marrow aspirate and biopsy revealed a cellular marrow with normal myelopoiesis and megakaryopoiesis. However, there was nearly complete absence of erythroid precursors. Rare pronormoblasts and basophilic erythroblasts were observed. There were no dysplastic or megaloblastic changes. Reticulin and iron stains were normal. Marrow cytogenetics showed a normal female karyotype. A diagnosis of pure red cell aplasia was made. Additional studies, including serum folate,  $B_{12}$ , direct and indirect Coomb's test, and sugar water tests were all normal or negative. A serum protein electrophoresis and quantitative immunoglobulins revealed a mild increase in polyclonal IgG but no monoclonal spike. Viral serologies revealed no evidence of recent or active parvovirus, hepatitis A, B, C, or Epstein-Barr virus infection. A serum erythropoietin level by radioimmunoassay revealed a level that was ap-

propriately elevated at 2,440 U/L, taken when the hemoglobin was 55 gm/L and after receiving a total of 24 units of packed red cells since initiation of chemotherapy. The chest radiograph and CT scans were normal. In vitro erythroid colony studies (see Results) were consistent with T cell mediated suppression of erythropoiesis.

The response to treatments are shown in Figure 1. No reticulocytosis was observed following cessation of all antibiotics. The patient was given a single course of i.v. immune globulin (400 mg/kg/dose; Sandoglobulin; Sandoz Pharmaceuticals, East Hanover, NJ) for 5 days (total dose 2 gm/kg). There was no evidence of a reticulocytosis after 4 weeks. On the basis of the invitro colony studies, the patient was started on cyclosporin A (Sandimmune; Sandoz Pharmaceuticals, East Hanover, NJ) at a dose of 6 mg/kg p.o. b.i.d. The dose was adjusted to maintain the serum cyclosporin level of 200–440 ng/mL. Immunophenotyping of peripheral blood lymphocytes before initiation of cyclosporin showed a depressed CD4/CD8 ratio of 0.87. There was a relative decrease in total T cells marking with CD2 ( $34 \times 10^6/\text{L}$ , normal range 66–246  $\times 10^6/\text{L}$ ) and in the CD4 helper T cell population ( $20 \times 10^6/\text{L}$ , normal range 41–148  $\times 10^6/\text{L}$ ). The patient tolerated the cyclosporin well. After 3 months of treatment, however, the patient failed to develop a reticulocytosis. Sequential erythropoietin levels drawn when the hemoglobin was between 55 and 108 gm/L showed a decline in erythropoietin levels to a nadir of 15 IU/L (normal 2–14 U/L). Cyclosporin was discontinued after 3 months. Repeat immunophenotyping studies following discontinuance of cyclosporin showed a correction of the CD4/CD8 ratio to 1.26 due to a relative increase in total T cells ( $110 \times 10^6/\text{L}$ ) and CD4 positive helper T cells ( $65 \times 10^6/\text{L}$ ). The patient was started on a trial of recombinant erythropoietin (Epogen; Amgen Inc, Thousand Oaks, CA) at a dose of 50 IU/kg i.v. TIW. Following initiation of recombinant human erythropoietin (rEPO) the reticulocyte count increased to a peak of 2.4%. Erythropoietin was continued for 10 months and then discontinued when the hemoglobin was stable at 100 gm/L. The patient became transfusion independent. An brief 2 month course of rEPO was later given when the hemoglobin fell to 82 gm/L. The patient has been able to maintain a stable hemoglobin of 100 gm/L in the absence of recombinant erythropoietin for the last 8 months. Platelet counts remained in the normal range and the white cell counts were  $4.4\text{--}9.4 \times 10^9/\text{L}$  with normal differential counts. The patient remains in a continuous complete remission from Hodgkin's disease 3 years after completion of chemotherapy.

## METHODS

Immunophenotyping studies on peripheral blood lymphocytes were performed using direct or indirect immunofluorescence using the following monoclonal antibod-

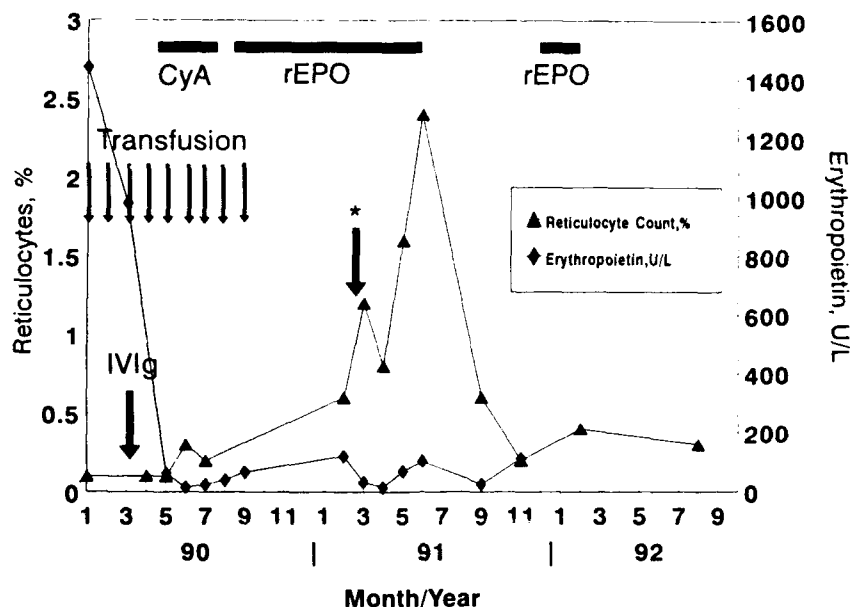


Fig. 1. Clinical course of a patient with pure red cell aplasia developing after treatment for Hodgkin's disease. Intravenous immunoglobulin (IVIg) was given at 40 g/kg/day for 5 days. Cyclosporin A was given at 6 mg/kg p.o. b.i.d. with the

dose adjusted to maintain a serum cyclosporin level of 200–400 ng/mL. Erythropoietin (rEPO) was initially given at 50 U/kg i.v. TIW and increased to (\*) 75 U/kg i.v. TIW. The patient received 3–4 units packed red cells during each transfusion.

ies: anti-Leu-4 (CD3), anti-Leu-3a (CD4), anti-Leu2a (CD8), anti-Leu-16 (CD20), anti-Leu-19 (CD56): Beckton-Dickinson, San Jose, CA; anti-T11 (CD2): Coulter Immunology, Hialeah, FL. In vitro erythroid colony studies for day 7 CFU<sub>E</sub> and day 14 BFU<sub>E</sub> were performed in a methocellulose culture system as previously described [9]. For day 7 CFU<sub>E</sub>, 0.5 IU/dish recombinant erythropoietin (Amgen, Thousand Oaks, CA) was added. 1.0 IU recombinant erythropoietin and 1.0 IU Interleukin-3 (Amgen) was present in each dish for day 14 BFU<sub>E</sub>. Target cells for these studies were the patient's light density mononuclear marrow cells. T cells were isolated by sheep erythrocyte rosetting techniques as previously described [9]. For serum studies, patient's serum at a final concentration of 10% was added directly to the plates in the presence or absence of complement (Pel Freeze Biologicals, Rogers, AK). For cyclosporin studies, all mononuclear bone marrow target cells were preincubated with varying doses of cyclosporin (Sandoz Pharmaceuticals, East Hanover, NJ) diluted in alpha minimum essential medium (Alpha MEM, GIBCO, Grand Island, NY). Student's two tailed t-test was employed to compare results between control and test cohorts.

## RESULTS

The effects on erythroid colony growth (day 7 CFU<sub>E</sub> and day 14 BFU<sub>E</sub>) of patient sera, autologous T cells, and cyclosporin are summarized in Table I; 127 ± 14 (mean ± ISD) day 7 CFU<sub>E</sub> and 23 ± 3 day 14 BFU<sub>E</sub>

were present in patient marrow and media controls and in absence of autologous serum. The addition of 10% autologous serum in the presence or absence of complement resulted in stimulation rather than inhibition of erythroid colony growth. Similar responses in CFU<sub>E</sub> (data not shown) were seen with heat inactivated patient serum as well as with normal type AB serum control (untreated, heat inactivated, or with the addition of complement). Removal of T cells from marrow whole mononuclear cells resulted in significant augmentation of erythroid colony growth in T cell depleted (TD) cells cultured alone (255 ± 23 CFU<sub>E</sub> ( $P < 0.01$ ) and 43 ± 6 BFU<sub>E</sub> ( $P = 0.06$ )) compared to media controls. The addition of either 10<sup>5</sup> or 2 × 10<sup>5</sup> autologous T cells to TD marrow reduced day 7 CFU<sub>E</sub> to 63% and 62% of the TD controls, consistent with T cell mediated suppression of erythropoiesis ( $P < 0.05$ ). BFU<sub>E</sub> growth decreased to 74% and 81% of TD controls ( $P > 0.05$ ). To determine whether cyclosporin would augment erythroid colony growth, patient's marrow whole mononuclear cells were preincubated with graded doses of cyclosporin in the range of 10 to 1,000 ng/mL for 1 hour, washed, and then replated. Compared to media controls, there was significant augmentation of CFU<sub>E</sub> in the 100–1,000 ng/mL cyclosporin range ( $P < 0.05$ ) and for BFU<sub>E</sub> at 10 and 500 ng/mL dose levels ( $P < 0.05$ ).

## DISCUSSION

The association of Hodgkin's lymphoma and PRCA is rare [4–8]. In two of the cases, an IgG inhibitor to eryth-

TABLE I. In Vitro Erythroid Colony Studies

Culture conditions <sup>a</sup>	CFU <sub>E</sub> <sup>b</sup>	% Control <sup>c</sup>	BFU <sub>E</sub> <sup>b</sup>	% Control
Serum studies <sup>d</sup>				
WMNC + media control	127 ± 14	—	23 ± 3	—
WMNC + 10% pt serum	319 ± 16 <sup>d</sup>	(251)	64 ± 4 <sup>d</sup>	(278)
WMNC + 10% pt serum (-C')	290 ± 14 <sup>d</sup>	(239)	102 ± 12 <sup>d</sup>	(443)
WMNC + 10% pt serum (+C')	245 ± 13 <sup>e</sup>	(211)	47 ± 2 <sup>e</sup>	(204)
T cell coculture studies				
TD alone	255 ± 23 <sup>d</sup>	(201)	43 ± 6 <sup>n.s.</sup>	(187)
TD + 2 × 10 <sup>5</sup> T cells	160 ± 3 <sup>e</sup>	(63)	32 ± 5 <sup>n.s.</sup>	(74)
TD + 10 <sup>5</sup> T cells	158 ± 12 <sup>e</sup>	(62)	35 ± 2 <sup>n.s.</sup>	(81)
Cyclosporin A studies				
WMNC + 10 ng/mL CyA	164 ± 6 <sup>n.s.</sup>	(129)	41 ± 5 <sup>e</sup>	(178)
WMNC + 100 ng/mL CyA	193 ± 4 <sup>e</sup>	(152)	38 ± 9 <sup>n.s.</sup>	(165)
WMNC + 500 ng/mL CyA	212 ± 6 <sup>e</sup>	(167)	39 ± 6 <sup>e</sup>	(169)
WMNC + 1,000 ng/mL CyA	197 ± 21 <sup>e</sup>	(155)	21 ± 4 <sup>n.s.</sup>	(91)

<sup>a</sup>2.0 × 10<sup>5</sup> patient marrow light density whole mononuclear cells (WMNC) or T cell depleted WMNC (TD) were plated in triplicate culture dishes with 0.5 IU erythropoietin for CFU<sub>E</sub> and 1.0 IU erythropoietin for BFU<sub>E</sub>. For coculture studies, 10<sup>5</sup> or 2.0 × 10<sup>5</sup> patient T cells were added to test for T cell suppression of erythroid colony growth. For serum studies, patient serum was tested at a final concentration of 10% in the presence or absence of complement (C'). For cyclosporin A (CyA) studies, patient marrow WMNC were preincubated with 10–1,000 ng/mL CyA for 1 hour, washed, and then plated.

<sup>b</sup>Values represent mean ± 1SD for triplicate culture dishes.

<sup>c</sup>Values in parentheses represent % media control (127 CFU<sub>E</sub> or 23 BFU<sub>E</sub>) for serum and cyclosporin studies and for TD alone. Controls for T cell coculture studies were expressed as %TD alone (255 CFU<sub>E</sub> or 43 BFU<sub>E</sub>).

<sup>d</sup>P ≤ 0.01 by Student's two-tailed t-test vs. control.

<sup>e</sup>P ≤ 0.05 by Student's two-tailed t-test vs. control.

<sup>f</sup>Similar responses in CFU<sub>E</sub> (data not shown) were seen with heat inactivated patient serum as well as with normal type AB serum control (untreated, heat inactivated, or with the addition of complement).

<sup>n.s.</sup> Not significant.

ropoiesis was implicated (Table II). In the present case, PRCA developed immediately following 6 months of multiagent chemotherapy with MOPP/ABV. In addition to exposure to these chemotherapeutic agents, the patient was exposed to multiple antibiotics, including a variety of penicillins, cephalosporins, gentamicin, trimethoprim-sulfamethoxazole (Bactrim), and pentamidine. PRCA has been associated with sulfamethoxazole [10,11] and possibly ampicillin [12]. We are not aware of any previous association of PRCA with any of the antineoplastic agents employed. It is unlikely in this case that Bactrim or other drugs were the cause of PRCA since discontinuance of the drugs did not result in the resolution of the anemia as has been observed in previous cases [10,11]. Additional studies excluded exposure to hepatitis, parvovirus or E-B virus known for causing PRCA [1]. Following the completion of the MOPP/ABV therapy and the development of a clinical remission, the patient developed a relative decrease in peripheral blood T cells and inversion of the normal CD4/CD8 (helper/suppressor) ratio. A variety of immunoregulatory disturbances have been previously reported in Hodgkin's disease, both before and after therapy [13–16]. The CD4/CD8 may be either depressed or normal at the time of diagnosis [14,15]. After completion of chemotherapy, the CD4/CD8 ratio is depressed if initially normal [16] or further depressed when initially low [15]. Taken together, these

studies suggest that the immunosuppressive effect of the MOPP/ABV therapy and/or Hodgkin's disease may have contributed to the emergence of autoreactive T cell erythroid suppressor cells and the subsequent development of PRCA.

The majority of cases of acquired PRCA that have been carefully studied are due to either T cell mediated suppression or antibody mediated inhibition of erythropoiesis [1]. Autoantibodies or autoreactive T lymphocytes may attack erythroid precursors (pronormoblasts) or erythroid progenitor cells (CFU<sub>E</sub> or BFU<sub>E</sub>). Natural killer (NK) cells have also been implicated in the development of PRCA [17–19]. Patients with lymphoproliferative disorders may apparently develop erythroid suppression by either T cell mediated or antibody mediated mechanisms [9,20]. T lymphocytes may suppress in vitro erythropoiesis in patients with chronic lymphocytic leukemia associated with PRCA [21–25] and some of these patients have become transfusion-independent following treatment with cyclosporin or antithymocyte globulin [21–24]. In the present case, we provided evidence for T cell mediated suppression of erythroid progenitor cell growth in vitro. There was no evidence for a serum inhibitor. In addition, in vitro preincubation of patient's marrow with cyclosporin showed a dose effect of cyclosporin on erythroid colony growth. Cyclosporin A may interfere with the function of activated T cells [26]. Following in vivo

TABLE II. Literature Review of PRCA Associated With Hodgkin's Disease

Reference	Age/sex	Histology	Mechanism	Stage	Treatment	Response to treatment	
						Lymphoma	PRCA
4 <sup>a</sup>	63/M	NR <sup>b</sup>	NR	NR	cortisone	CR <sup>c</sup>	CR
5 <sup>a</sup>	36/F	NR	Ig <sup>d</sup>	NR	RT/VCR <sup>e</sup>	TR <sup>f</sup>	TR
6 <sup>a</sup>	16/M	MC <sup>g</sup>	Ig(G) <sup>h</sup>	IVA	MOPP	CR	CR
7 <sup>a</sup>	19/M	LD <sup>i</sup>	NI <sup>j</sup>	IVB	CDVP <sup>k</sup>	CR	CR
8 <sup>a</sup>	60/M	MC	NR	IIIB	MOPP	CR	CR
This paper <sup>l</sup>	24/F	NS <sup>m</sup>	T cell	IIIB	MOPP/ABV	CR	—

<sup>a</sup>PRCA noted at diagnosis of Hodgkin's disease.

<sup>b</sup>Not reported.

<sup>c</sup>Complete response.

<sup>d</sup>Immunoglobulin mediated. Ig class not specified.

<sup>e</sup>Radiation therapy and vincristine.

<sup>f</sup>Transient response.

<sup>g</sup>Mixed cellularity.

<sup>h</sup>IgG mediated.

<sup>i</sup>Lymphocyte depleted.

<sup>j</sup>Not immunoglobulin mediated (probably T-cell mediated).

<sup>k</sup>Cytoxan, doxorubicin, vincristine and prednisolone.

<sup>l</sup>PRCA developed after treatment for Hodgkin's disease.

<sup>m</sup>Nodular sclerosing.

administration of cyclosporin, our patient had a dramatic increase in T helper cells with normalization of the CD4/CD8 ratio. Collectively, the in vitro studies and in vivo T cell subset response to the administration of cyclosporin implicates T cells in the pathogenesis of the PRCA in our patient.

Although the weight of the evidence was consistent with T cell mediated suppression in this case, the patient did not develop an immediate reticulocytosis during a 3 month trial of cyclosporin. Cyclosporin is well known to impair erythropoietin production in humans [27-29] and in mice [30]. As was documented, inappropriately low erythropoietin levels developed in our patient following cyclosporin administration. Upon institution of subcutaneous erythropoietin, a brisk reticulocytosis was seen and a sustained erythropoietic response was subsequently observed in the absence of both erythropoietin and cyclosporin.

## CONCLUSION

It is likely that the MOPP/ABV therapy and/or the Hodgkin's disease induced and immunoregulatory disturbance, allowing the emergence of autoreactive erythroid suppressor T cells which triggered the PRCA in our patient. The immediate response to cyclosporin was masked by the development of impaired erythropoietin production. Correction of this problem resulted in a dramatic reversal of the anemia. Erythropoietin levels should be monitored in patients with PRCA or aplastic anemia receiving cyclosporin if there is no reticulocytosis. The addition of subcutaneous erythropoietin concomitantly or

following cyclosporin may unmask a defective response due to inappropriate erythropoietin levels.

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